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Commentary

Antigen-specific immunotherapy and influenza vaccination in type 1 diabetes: timing is everything

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Abbreviations

ASI	Antigen-specific immunotherapy
HIRD	Human Immune Response Dynamics
Th	T helper

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Introduction

Antigen-specific immunotherapy (ASI) has long been hailed as a promising strategy to induce or restore immune tolerance in beta cells, the loss of which underlies the development of type 1 diabetes. The approach is based on the observation that, in preclinical models, repeated administration of an antigen over days or weeks leads to a state of immunological unresponsiveness. When the antigen used is a major target of the immune response in an autoimmune disease, the result is tolerance and disease prevention.

In the type 1 diabetes space, there are several efforts under development to realise the potential benefits of ASI, including daily oral insulin, peptide antigen immunotherapy (e.g. proinsulin peptide) and GAD conjugated to alum [1-4]. GAD is well established as an important autoantigen in type 1 diabetes, and anti-GAD antibodies and GAD-reactive T cells are early features of the disease process. GAD-alum is an ASI strategy designed to induce T helper (Th) 2 counter-regulation of the pathological Th1 autoimmune process that is thought to underlie beta cell destruction. Manipulation of such autoimmune networks, which may be finely balanced between health and disease, presents an important opportunity to learn about self-reactivity and how it may be influenced. In the current issue of *Diabetologia*, Tavira et al describe such an opening [5].

GAD-alum for the prevention of beta cell loss in type 1 diabetes

The potential therapeutic effect of GAD-alum in preventing beta cell loss in individuals with a recent diagnosis of type 1 diabetes has been evaluated in several studies, from Phases I to Phase III. Whilst there is clear evidence that GAD-alum increases GAD autoantibody levels and induces GAD-specific Th2 responses, the clinical results have been somewhat equivocal [1, 6-8]. The pivotal study is a Phase III GAD-alum trial in individuals with new-onset type 1 diabetes, which failed to meet its primary efficacy endpoint of preserving C-peptide production [9]. However, important subgroup analyses of this study revealed that beneficial effects of GAD-alum use were indeed observed in individuals from non-Nordic countries, but not in the Nordic participants who comprised nearly half of the study cohort. The study by Tavira et al [5] provides data to argue that this discrepant outcome is not a result of geographical differences but of different influenza protection strategies in the various countries participating in the clinical trial.

Influenza vaccination and GAD-alum efficacy The Phase III trial protocol prohibited the use of routine infectious-disease vaccines around the study period (<1 month before and <2 months after the first dose of GAD-alum). This is not uncommon in immunotherapy trials and is used as a means to avoid unknown adverse interactions with ASI or, in the case of immunotherapies with suppressive properties, to avoid reduced vaccine efficacy. Vaccine exclusion can be challenging to police, not least because of guidelines that individuals with diabetes should be vigilant in maintaining active immunity through vaccination, including annual national programmes for influenza prevention. In the case of the GAD-alum Phase III study, recruitment coincided with an H1N1 influenza virus pandemic; hence, under these

circumstances, influenza vaccination was permitted. The majority of Swedish and Finnish participants were vaccinated with Pandemrix, which was not in use in non-Nordic countries. This prompted Tavira and colleagues to examine whether Pandemrix use could have impacted upon GAD-alum immunotherapy outcomes [5]. The authors chose to address this by examining the effects of influenza vaccination on outcome measures in relation to the timing of Pandemrix administration. Participants were divided according to whether influenza vaccination was given less than 5 months (<150 days) or 5–15 months before or after the first dose of GAD-alum. Individuals with a longer period between influenza vaccination and GAD-alum administration had higher GAD autoantibody levels, higher GAD-induced Th2 cytokines and reduced C-peptide decline compared with those who were vaccinated close to GAD-alum therapy (as summarised in Fig. 1). The authors conclude that if Pandemrix is given too soon before or after GAD-alum injection, it may interfere with the desired clinical and mechanistic effects of ASI.

The impact of influenza vaccination on GAD-specific immune responses

Alum was selected as an adjuvant for GAD in order to steer away from a Th1-dominated, proinflammatory cellular autoimmune response towards a Th2 response, which favours humoral immunity (antibody production) [10]. Tavira et al demonstrated that individuals who received the influenza vaccination within close proximity of GAD-alum treatment had increased secretion of GAD-induced proinflammatory cytokines, such as IFN-γ and TNF-α, compared with those who received the vaccine further from GAD-alum therapy [5]. One speculation is that the increase in proinflammatory cytokines may have contributed to the accelerated C-

peptide loss in the participants vaccinated <5 months before or after GAD-alum treatment. Conversely, an IL-13 dominated GAD-induced profile was observed in individuals with a 5–15 month gap between GAD-alum treatment and influenza vaccination. This Th2 skewing represents the anticipated response to GAD-alum and may have contributed to C-peptide retention in this group. It is also possible that the enhanced GAD-specific Th2 response in these individuals may have accounted for the increased humoral GAD-specific immunity observed in these participants; importantly, this observation fits with a previous report of elevated GAD antibodies in individuals treated with GAD-alum in a Phase III study [11]. However, there are some caveats to the study by Tavira et al; for example, there are no placebo comparisons and, therefore, it remains unknown whether the group vaccinated with Pandemrix >5 months before or after GAD-alum benefitted from GAD-alum as a therapeutic agent or merely avoided any non-specific adverse effects of Pandemrix on beta cell destruction pathways (see Fig. 2) that are more active in the period soon after diagnosis.

Intriguingly, differences in the GAD-specific immune response and C-peptide in participants vaccinated with the H1N1 vaccine and GAD-alum <5 months or >5 months apart were only observed in the individuals who received two doses but not four doses of GAD-alum. It is plausible that four doses of GAD-alum is sufficient to overcome any modulatory effects of Pandemrix that were observed in the two-dose group. The authors also noted that they had previously observed greater C-peptide retention in individuals who received two doses vs four doses of GAD-alum [12]. Furthermore, levels of H1N1 antibodies were significantly reduced in individuals

vaccinated with Pandemrix close to receiving GAD-alum in the group receiving four doses of the influenza vaccine, implying that responses to both GAD and influenza were reduced as a result of the close proximity of the two interventions.

Cellular mechanisms for GAD-alum/influenza vaccine interference

This study raises the possibility that influenza vaccination interferes with ASI, a phenomenon that is both intriguing from a mechanistic standpoint and worrying from a clinical and drug development vantage. It is known that the co-administration of vaccines can result in immunological interference [13] and it will be of interest to understand how such interference mechanisms apply in the setting of ASI. Sequential administration of vaccines can result in the suppression of a response to one antigen at the expense of another or reduced responses to both antigens. Such effects depend on the relative amounts of antigens administered, the relative sites of vaccination and the time interval between antigen administration. For example, an impaired response to one antigen over another upon co-administration of vaccines may arise as a result of competition between multiple antigen responses for limited resources within the lymph nodes, such as access to antigen, chemokines, activation signals, follicular dendritic cells and follicular Th cells, thereby affecting the level of T cell help, B cell activation and antibody production directed towards a given antigen. Alternatively, co-administered vaccines may change the balance of Th cell subsets, since different antigens may induce mutually antagonistic Th1 or Th2 cytokine responses, or induce regulatory T cell mechanisms that could inhibit the immune response to one of the antigens administered.

Tavira and colleagues speculate that the interference between influenza vaccination and GAD-alum administration observed in this particular setting could be attributable to the potent immunomodulatory effects of the adjuvant used in Pandemrix, AS03 [5]. AS03 has been shown to enhance the vaccine's antigen-specific adaptive response by locally activating the innate immune system and by increasing antigen uptake and presentation in draining lymph nodes [14]. Since adjuvants act directly as immune stimulants, there is a possibility that they induce undesirable immune responses that could trigger the onset of immune-mediated disease in susceptible individuals. Indeed, following vaccination with Pandemrix during the 2009 H1N1 pandemic, some European countries, including Sweden and the UK, reported the emergence in a small number of cases of narcolepsy, an immune-mediated destruction of hypocretin-secreting neurons in the hypothalamus. Pandemrix elicits a transient, rapid and expansive activation of myeloid cells and effector cells, similar to the responses induced by other vaccines, including non-adjuvanted influenza vaccines [15-20]. However, it also differs from other vaccines; the Human Immune Response Dynamics (HIRD) study in our laboratory showed that Pandemrix provokes an overt early-lymphoid response within 24 h of vaccination, with prominent upregulation of IFN-γ transcription, which has not been observed in most other virus vaccine studies [16]. Early-lymphoid- and myeloid-dominated responses were followed by a plasmablast-dominated response at day 7. Therefore, in participants vaccinated with Pandemrix soon before or after starting GAD-alum treatment, the notion that Pandemrix could affect GAD-associated immunity is, perhaps, not surprising, given that the outcome of auto-immunisation depends on the pre-existing immune background. For example, in the absence of inflammatory

stimuli, an increased production of TGF-β and other immunomodulatory cytokines in response to Pandemrix may favour the generation of a tolerogenic response to administered autoantigen. On the other hand, a pre-existing pool of activated effector T cells and an aggressive proinflammatory response initiated by Pandemrix might instead aggravate autoimmunity and even accelerate autoimmune beta cell destruction.

Intriguingly, the HIRD study also found that adverse events arising from Pandemrix vaccination were associated with an atypical pre-vaccination B lymphocyte phenotype, with an expanded transitional B lymphocyte pool (which is seen in autoimmunity) and higher levels of autoantibodies [16]. This observation might suggest that non-specific immune activation is enhanced in individuals with an autoimmune background, and a similar phenomenon might have contributed to the effects of Pandemrix in the GAD-alum study.

Application of findings

An important issue will be how this set of serendipitous observations from Tavira et al can and should be built upon. The first question is whether greater mechanistic insights can be gained in future studies, and the second is how it will impact upon the design of ASI trials. It is likely that new studies in man to address the many arising mechanistic questions are precluded by safety and feasibility issues and preclinical models may not be helpful in this setting. To further complicate matters, Pandemrix is no longer in use. Perhaps the best that can be achieved is to obtain

robust vaccine record data in ASI studies and consider relevant sub-analyses in relation to study outcomes. What are the other implications for the design of ASI trials in type 1 diabetes? Perhaps it is reasonable for routine infectious-disease vaccines to be avoided for a period of 6 months before or after the ASI treatment period. However, in some settings (e.g. The Diabetes Prevention Trial of Type 1 Diabetes [DPT-1], an oral insulin study; ClinicalTrial.gov registration no. NCT00004984) antigen administration is continuous over months/years and withholding vaccines is neither practical nor desirable. In addition, it is probable that natural exposures to infectious agents could have adverse effects on ASI and these remain beyond easy control. The study by Tavira et al highlights the need to consider the immune background on which ASI is administered more generally and emphasises the yin and yang of immune responsiveness in seeking to balance aggression and regulation in the face of complex external stimuli.

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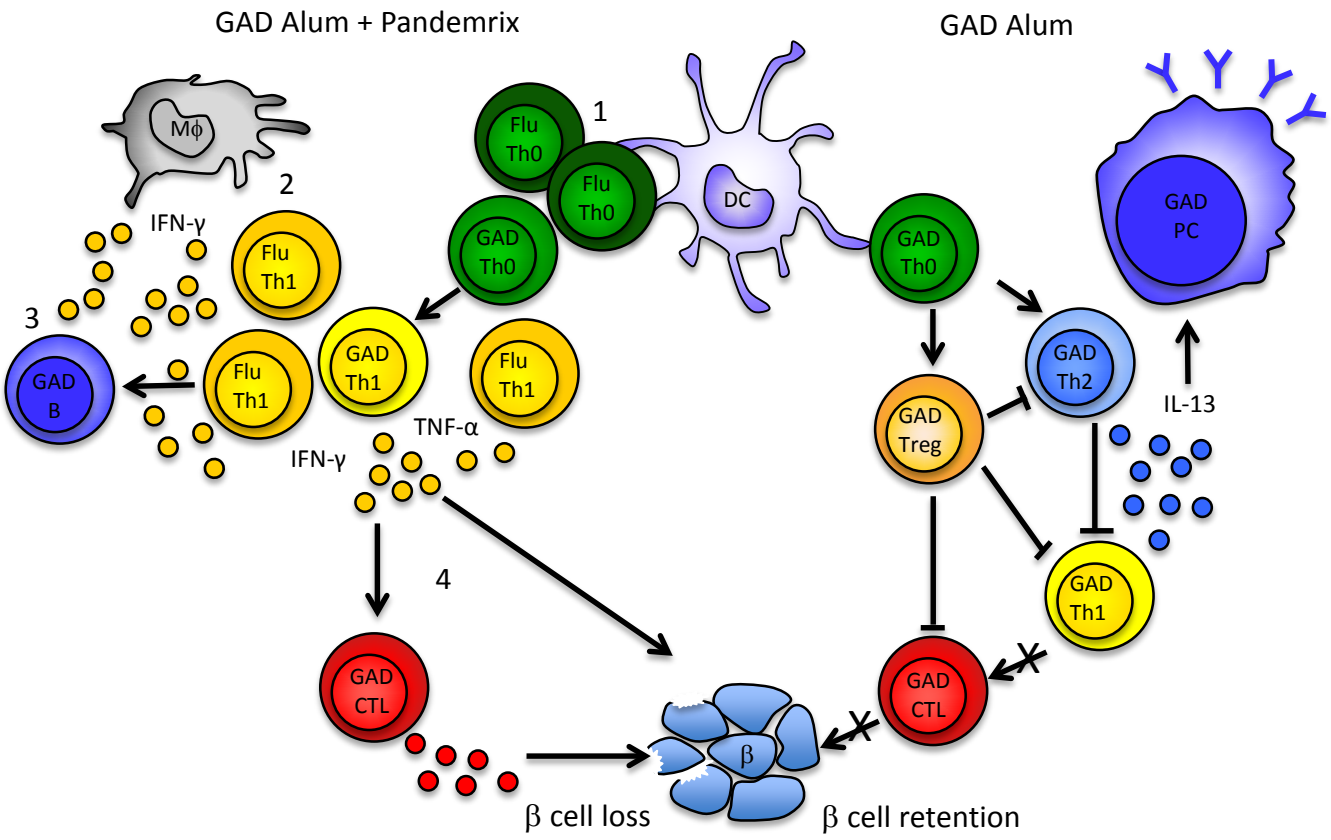
Fig. 1 Outcome of two doses of GAD-alum immunotherapy differs according to whether the Pandemrix influenza vaccination is administered <150 days or >150 days from first GAD-alum dose. GADA, GAD autoantibodies

Fig. 2 Mechanisms by which Pandemrix may interact with GAD-alum immunotherapy. In the absence of Pandemrix, GAD-alum induces a Th2 response that counterbalances and limits destructive Th1-mediated responses. Pandemrix vaccination close to GAD-alum administration may result in the following scenarios: (1) competition between GAD and influenza (flu) responses in the lymph node; (2) elevated IFN-γ levels resulting from the Pandemrix-induced myeloid and lymphoid response create a proinflammatory environment and may thus promote a GAD-specific Th1 response; (3) Th1 cytokine-activated B cells may contribute to the proinflammatory cytokine milieu; (4) Th1 cytokines may drive beta cell death directly and via cytotoxic T cells. β, beta cell; B, B cell; CTL, cytotoxic T lymphocyte; DC, dendritic cell; Mϕ, macrophage; PC, plasma cell; Treg, regulatory T cell


GAD Alum + Pandemrix
GAD Alum
Mϕ
2
IFN-γ
Flu Th1
3
GAD B
Flu Th1
GAD Th1
Flu Th1
TNF-α
IFN-γ
4
GAD CTL
β cell loss
Flu Th0
1
Flu Th0
DC
GAD Th0
GAD Th0
GAD PC
GAD Th2
IL-13
GAD Treg
GAD Th1
GAD CTL
β
β cell retention